Stem Cell Expansion in a Fluidised Bed Bioreactor for Accelerated Osseointegration of Bone Substitute Material

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Introduction

The ultimate aim of this work is to expand human mesenchymal stem cells within porous bioceramic particles, in a fluidised bed bioreactor, and use the cell-particle constructs to accelerate osseointegration of the material with bone. The patented particles (patent no. US 8,101,268) have been fabricated with interconnected pores of 150 – 300 µm for optimal bone ingrowth [1,2]. The computational fluid dynamic package FLUENT is being used to inform bioreactor geometry and operating conditions (flow rates, pressures, seeding densities, solute concentrations *etc*.) and aid the development of operating equations. Here we present two steps towards this aim: a comparison of experimental and CFD fluidisation of BoneSave, a commercial precursor to our particles, and quantification of shear stress required to detach cells from tissue culture plastic.

Materials and Methods

Experimental and CFD Modelling of Ceramic Particle Fluidisation

Fluidisation experiments and modelling were performed using the BoneSave particles in water. The particles were 2-4 mm in diameter with a density of 2200 kgm^{-3} (Figure 1). These irregular particles with 50% isolated porosity were investigated as a stepping-stone between solid spheres, which are the standard geometry used in fluidised bed systems, and our irregular and highly-porous particles. The BoneSave particles were modelled as solid spheres with a diameter of 3 mm and a density of 2200 kgm^{-3}. The fluidised bed bioreactor had a height (h) of 0.1 m and diameter of 0.025 m. The range of velocities (v) analysed was 0.030, 0.035, 0.048 and 0.071 ms^{-1}. The bed comprised approximately 800 particles.

Figure 1: BoneSave particles.

The FLUENT modelling was performed using the 'dense discrete phase' model available as one of the Eulerian multiphase options. This entailed modelling the water phase as an Eulerian continuum and the solid phase as a set of explicitly modelled individual particles. Mesh length was set to 4 mm, the velocity inlet was a uniform inlet averaging the quoted velocities. The first time step included the injection of 192 particles to form the bed.

Shear Stress of Detachment from Tissue Culture Plastic

A convergent flow chamber was designed to enable a range of shear stresses to be analysed in a single experiment. The base of the chamber was the bottom internal cell-culture surface of a standard T75 flask. The chamber had a decreasing cross-sectional area along its length such that the linear velocity and therefore shear stress increased from the inlet to the outlet of the chamber. The chamber width decreased from 54 mm at the inlet to 14 mm at the outlet, over a distance of 50 mm.

The chamber was sterilised overnight with 5% penicillin / streptomycin solution in PBS prior to seeding the osteosarcoma cell line MG63 at a density of 20,000 cells/cm^2 by injecting the cell suspension in complete media (DMEM containing 10% (v/v) foetal calf serum (FCS)). Static conditions were maintained for 6 or 24 hours to allow for attachment before carrying out the shear stress experiments. The chamber was maintained at 37°C and 5% CO_2.

Experiment 1. Six-hour attachment. Phosphate buffered saline (PBS) for 20 minutes using two flowrates (Q). Q = 80 ml/min for 10 minutes then Q = 150 ml/min for 10 minutes. These flowrates were selected after initial tests showed the cells were detached in this range.

Experiment 2. Six-hour attachment. PBS for 60 minutes, Q = 28 ml/min with analysis after 10 minutes, 20 minutes and 30 minutes consecutively, for a total of 60 minutes. This flowrate was selected because Experiment 1 showed that minimal numbers of cells remained attached at shear stresses of 100 mPa and higher.

Experiment 3. 24-hour attachment. Complete media for 30 minutes at Q = 560 ml/min. The flowrate was gradually increased to this value at the start of the experiment, it was the maximum flowrate the chamber could contain at that time.

The shear stress of the fluid was calculated at the mid-point of the flow channel. In each experiment light micrographs were recorded after each step, the number of adhered cells in each micrograph was counted manually and the position within the chamber, and thus the associated shear stress, recorded.

Results and Discussion

Experimental and CFD Modelling of Ceramic Particle Fluidisation

Prior to fluidisation the particles form a packed bed on the distributor. The initial bed height was 0.03 m for the experimental model and 0.02 m for the CFD model (Figure 2a and 3a respectively). When v = 0.035 ms^{-1} the drag forces became sufficiently large to overcome gravity so each particle was just suspended and an expanded bed formed. The bed height for the experimental model was 0.04 m, and 0.03 m for the CFD model (Figure 2b and 3b respectively). At v = 0.071ms^{-1} the bed is fully fluidised (Figure 2c and 3c). The experimental bed height was 0.08 m and the CFD bed height was 0.04 m. There are two likely reasons for the discrepancies: the particles were approximated as solid spheres of a uniform diameter, whereas the experimental bed comprised irregular shaped particles varying between 2 and 4 mm in diameter. The smaller particles will have fluidised at a lower velocity. Modelling the inlet with a uniform flow distribution akin to plug flow, as opposed to that obtained with a distributor will also have affected the model.

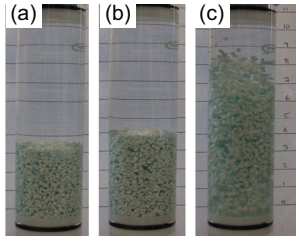

Figure 2: The experimental model of the stages of fluidisation of BoneSave. Bed height increases with increasing velocity. (a) v = 0.030 ms^{-1}, h = 0.03 m, (b) v = 0.035 ms^{-1}, h = 0.04 m, (c) v = 0.071 ms^{-1}, h = 0.08 m.

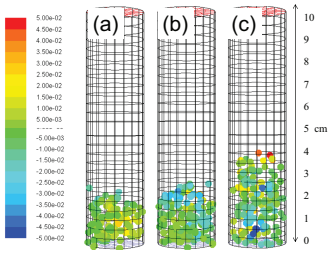

Figure 3: The FLUENT model of the stages of fluidisation of BoneSave. (a) v = 0.030 ms^{-1}, h = 0.02 m, (b) v = 0.035 ms^{-1}, h = 0.03 m, (c) v = 0.071 ms^{-1}, h = 0.04 m. The colour gradient shows the velocity of the individual particles, in the vertical direction.

Shear Stress of Detachment from Tissue Culture Plastic

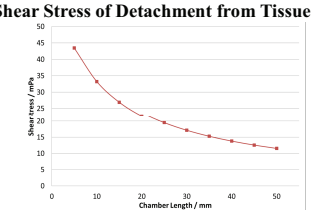

Figure 4: Wall shear stress as a function of chamber length. Q = 80 ml/min.

An example of the shear stress profile on the culture surface, i.e. the wall shear stress, as a function of chamber length is shown in Figure 4, at Q = 80ml/min. The shear stress ranges from 11.4 to 43.4 mPa, demonstrating a 3.8 fold increase along the chamber length.

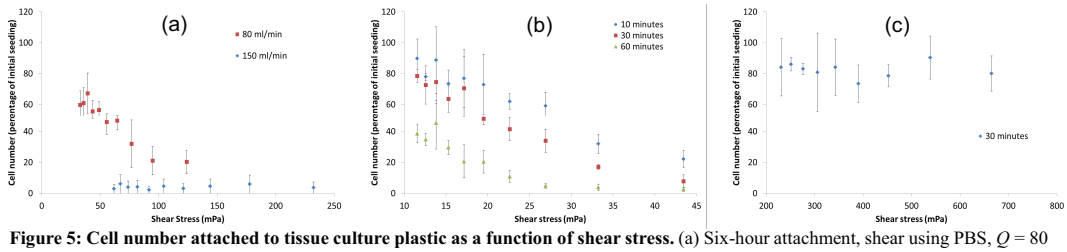

Figure 5: Cell number attached to tissue culture plastic as a function of shear stress. (a) Six-hour attachment, shear using PBS, Q = 80 ml/min for 10 min and Q = 150 ml/min for 10 min; (b) Six-hour attachment, shear using PBS, Q = 28 ml/min for 60 min; (c) 24-hour attachment, shear using complete media, Q = 560 ml/min for 30 min. Error bars are ± 1 s.d., n = 3.

The 80 ml/min data (Figure 5a) shows a decrease in cell number as the shear stress increases, whereas the 150 ml/min data shows a constant, very low, number of cells. The region where the two shear stress ranges overlap (25 -125 mPa) would show similar numbers of cells if the detachment was only dependent upon the shear stress, this data shows it is also time dependent.

The data shown in Figure 5b supports this as for each time period under a flow of 28 ml/min, successively more cells have been removed. Each set shows a similar pattern of decreasing cell number with shear stress, with the 60 minutes data levelling off at the same minima as the 150 ml/min data in Figure 5a. Cells seeded for 24 hours showed a similar pattern (data not shown). Therefore it can be concluded that the cells sheared with PBS are dependent on both the shear stress applied and the time exposed to shear.

When complete media was used for the shear tests the cells are less easily detached (Figure 5c). The number of cells is maintained at a relatively constant value of between 75% and 90% independent of the shear stress, ranged between 230 and 670 mPa. This is because of the presence of Ca^{2+} and Mg^{2+} ions, absent in the PBS, which are required for integrin-ligand binding [3]. It was observed that many of the cells in this test presented a flattened morphology and extending filopodia, whereas all cells sheared with PBS had a spherical morphology.

Conclusions and Future Work

- The fluidisation of dense calcium phosphate material has been successfully performed.
- A CFD model of the fluidisation has been successfully produced in FLUENT and the behaviour of the particles is similar to that of the particles in the experimental model. Adaptation to make the model fit the experimental data more accurately will be performed before the model is progressed to incorporate porous particles.
- A flow chamber has been successfully designed and used to provide a varying shear stress to a uniformly seeded surface.
- The adhered cells sheared with PBS were seen to be detached by shear stresses less than 50 mPa, whereas cells sheared with complete media were seen to remain attached at shear stresses up to 670 mPa. It is postulated that this difference is due to the absence of calcium and magnesium ions in the PBS buffer, further work will be performed to confirm this finding.
- The detachment of cells sheared with PBS was shown to be time dependent, whereas the cells sheared with complete media maintained a relatively constant value.
- The morphology of the cells sheared with PBS was seen to be spherical with no filopodia. A significant number of cells sheared with full media maintained the flattened morphology and filopodia seen with static growth.
- The highest linear velocity used on the cells in the shear stress experiments was 0.3 ms^{-1}. The particles used in the fluidised bed were shown to fluidise at a velocity below 0.1 ms^{-1}, therefore it can be hypothesised that the cells will remain adhered during fluidisation.

Refs: 1. Gittings, J.P. *et al.* Key Eng. Mater. **284-286**, pp. 349-352 (2005) 2. Hsu, Y.H. *et al.* J. Materials Sci.: Mat. in Med. **18**, pp. 1931-1937 (2007) 3. Lu, H. *et al.* Anal. Chem. **76** pp. 5257-5264 (2004)

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